AD

EDGEWOOD ARSENAL

TECHNICAL REPORT

EATR 4053

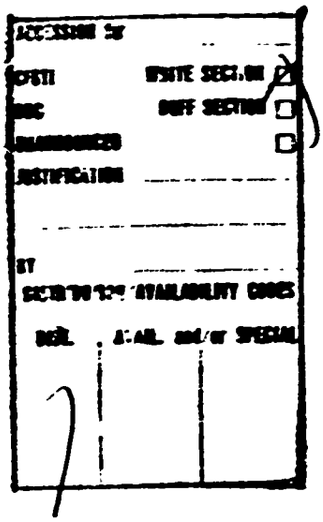
REACTIVATION BY 2-PAMCI OF INHIBITED ChE ACTIVITY IN DOGS POISONED WITH PINACOLYL METHYLPHOSPHONOFLUORIDATE (SOMAN, GD)

by

Joseph H. Fleisher
Larrel W. Harris
Edmund F. Murtha

January 1967

Medical Research Laboratory
Research Laboratories
EDGEWOOD ARSENAL
EDGEWOOD ARSENAL, MARYLAND 21010



Distribution Statement

Distribution of this document is unlimited.

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Disposition

When this report is no longer needed, destroy it. Do not return it to the originator

EDGEWOOD ARSENAL TECHNICAL REPORT

EATR 4053

REACTIVATION BY 2-PAMCl OF INHIBITED ChE ACTIVITY IN DOGS POISONED WITH PINACOLYL METHYLPHOSPHONOFLUORIDATE (SOMAN, GD)

by

Joseph H. Fleisher
Larrel W. Harris
Edmund F. Murtha

Physiology Department

January 1967

Task 1C622401A09709

Medical Research Laboratory
Research Laboratories
US ARMY EDGEWOOD ARSENAL
EDGEWOOD ARSENAL, MARYLAND 21010

FOREWORD

The work described in this report was authorized under Task 1C622401A09709, Prophylaxis and Therapy of Agent GD (U). The experimental data are contained in notebook MN-1847. This work was started in April 1964 and completed in May 1965.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

DIGEST

In this work, the effect of varying concentrations and dosages of 2-pyridine aldoxime methochloride (2-PAMCl) on the aging of cholinesterase (ChE) phosphonylated by soman in vitro and in vivo in dogs was studied.

The rate of aging of dog red-blood-cell (RBC) ChE inhibited by soman in vitro and in vivo can be markedly diminished by 2-PAMCl, which reactivates the unaged portion of the inhibited ChE enzyme. Measurements of brain- and diaphragm-ChE activity in dogs poisoned with soman and treated with 0.104 gm/kg of 2-PAMCl showed that reactivation occurred only in the latter tissue. The close correlation found between in vitro and in vivo rates of aging suggests that, in a given species, estimation of the rate of aging of RBC ChE in vitro following inhibition with an organophosphate could determine the time during which oxime therapy would be effective in vivo against intoxication by the same anti-ChE.

CONTENTS

LIST OF FIGURES

Figure

LIST OF TABLES

Table

REACTIVATION BY 2-PAMCl OF INHIBITED ChE ACTIVITY IN DOGS POISONED WITH PINACOLYL METHYLPHOSPHONOFLUORIDATE (SOMAN, GD)

I. INTRODUCTION.

When cholinesterase (ChE) enzyme is inactivated by organophosphates, it is progressively converted to a form that cannot be reactivated by nucleophilic compounds.[1-3] This conversion has been designated "aging" (loss of reactivatibility). A study of butyrylcholinesterase (BuChE) inhibited with diisopropyl phosphorofluoridate (DFP) in vitro by Berends and coworkers[4] showed that conversion of the inhibited enzyme to a nonreactivatible state is accompanied by a parallel loss of one isopropoxy group from the phosphorylated enzyme. The loss of the alkoxy constituent promotes a negative charge on the phosphonate bound to ChE, which results in resistance to nucleophilic attack by oximes whose active species is the oximate anion.[5] Later, Loomis and Salafsky[6] reported that soman produced an inhibited acetylcholinesterase (AChE) in vitro that was only slightly reactivated by oximes. In explanation, Fleisher and Harris[7] demonstrated that the very rapid aging of AChE inhibited by P^{32}-soman in vitro was closely correlated with the loss of a pinacolyl group from the phosphonylated enzyme. Fleisher and Harris[7] also observed that a high concentration of oxime could reactivate the unaged portion of AChE phosphonylated by P^{32}-soman. This finding led to the present work—to study the effect of varying concentrations and dosages of 2-PAMCl on the aging of ChE phosphonylated by soman in vitro and in vivo. The dog was chosen as the experimental animal because an adequate number of blood samples could be taken conveniently at rapid intervals after poisoning with the organophosphate.

II. MATERIALS.

Soman of 95% purity was obtained from the Chemical Process Division of Edgewood Arsenal.

A standard solution of 2-pyridine aldoxime methochloride (2-PAMCl) (obtained from the Aldrich Co., Milwaukee, Wisconsin), was prepared by dissolving 2-PAMCl in a solution of 0.1 M phosphate–0.9% NaCl at pH 7.6 to give a final oxime concentration of 1.25×10^{-1} M. The pH was again adjusted to 7.6 by the addition of a few drops of NaOH solution.

III. METHODS AND RESULTS.

A. Aging of Dog RBC ChE After Inhibition With Soman in Vitro.

Eight milliliters of heparinized whole blood were added to 32 ml of ice-cold 0.9% NaCl buffered to pH 8.8 with 0.01 M borate buffer. The mixture was incubated with 10^{-7} M soman for 15 min at 0°C. Preliminary experiments showed that virtually complete inhibition of ChE with minimal aging occurred under these conditions. The sample was centrifuged at 2000 rpm for 5 min at 2°C. The supernatant was discarded, and the red blood cells (RBC) were placed in a bath at 37°C; 3 min sufficed to equilibrate the temperature of the RBC to the bath temperature. A 0.3-ml aliquot of packed RBC was immediately transferred into the standard 2-PAMCl solution buffered at pH 7.6 with 0.1 M phosphate buffer so as to minimize aging during reactivation. A second aliquot of packed RBC was promptly placed in phosphate-buffered 0.9% NaCl without 2-PAMCl to serve as an inhibition control. Rapid aging of the remaining RBC ChE was immediately started by adjusting the pH to 7.35 with 4 ml of 0.05 M phosphate buffer in 0.9% NaCl (previously equilibrated to 37°C) for each milliliter of RBC. Based upon the observation that 10^{-1} M 2-PAMCl immediately stopped the aging of soman-inhibited ChE,* 2-ml aliquots of the suspension of RBC ChE undergoing aging were transferred into equal volumes of 2×10^{-1} M 2-PAMCl solution. The mixtures were incubated for 60 min at 25°C, a time sufficient for maximal reactivation of unaged ChE under these conditions. The RBC were centrifuged, and the supernatant was discarded. Five additional cycles of washing with 10 volumes of 0.9% saline and centrifuging followed, the supernatant being discarded each time. RBC not treated with soman and incubated with the 2-PAMCl solution were used as an additional control. The ChE activity of the dog RBC was measured colorimetrically, as described by Fleisher and coworkers.[8] The percentage of reactivated ChE was calculated from the formula suggested by Hobbiger[9]:

$$\%\ \text{of ChE reactivated} = \left(\frac{\text{ChE activity of oxime-treated phosphonylated tissue} - \text{ChE activity of inhibited controls}}{\text{ChE activity of oxime-treated controls} - \text{ChE activity of inhibited controls}} \right) \times 100$$

* Fleisher, J. H., and Harris, L. W. Unpublished observation.

From the in vitro data, the logarithms of the percentage of reactivated RBC-ChE activity were plotted as a function of time of aging prior to transfer into the 2-PAMCl solution (figure 1). The results are consistent with first-order kinetics. Additional trials with RBC ChE from seven dogs yielded similar results. The mean half times for aging were interpolated from each of the curves and used to calculate the rate constants shown in table I.

Table I. Aging of Dog RBC-ChE Activity After Inhibition With Soman in Vitro and in Vivo

Type of study*	Mean half time	Rate constant
	min	min^{-1}
In vivo	4.54 (3.82 - 5.26)**	0.139 (0.116 - 0.162)
In vitro	5.38 (4.73 - 6.03)	0.130 (0.119 - 0.141)

* Two groups of 8 dogs each were used for the in vitro and in vivo studies, respectively.

** Confidence limits, 95%, in parentheses.

B. Aging of Dog RBC-ChE Activity After Inhibition With Soman in Vivo.

The rate of aging of RBC ChE phosphonylated by soman in vivo was studied in eight dogs, who were anesthetized with 30 mg/kg of sodium pentobarbital and artificially respired. Atropine sulfate, 1 mg/kg, was injected intramuscularly about 20 to 30 min before the administration of soman. The left external carotid artery was cannulated to facilitate rapid withdrawal of blood samples. Before injection of soman, samples of approximately 1.5 ml were withdrawn and put into 9.5 ml of heparinized 0.9% NaCl buffered at pH 7.6 with 0.1 M phosphate buffer or into a separate tube containing the same solution with sufficient 2-PAMCl to yield a final oxime concentration of 10^{-1} M as controls. A 30-μg/kg dose of soman (approximating a dose of 3 LD50's) was injected intravenously into each dog via the jugular vein. Blood samples were then put into the 2-PAMCl solution and, within 10 sec, into phosphate-saline solution alone for the corresponding inhibition control. The samples were then processed as described previously.

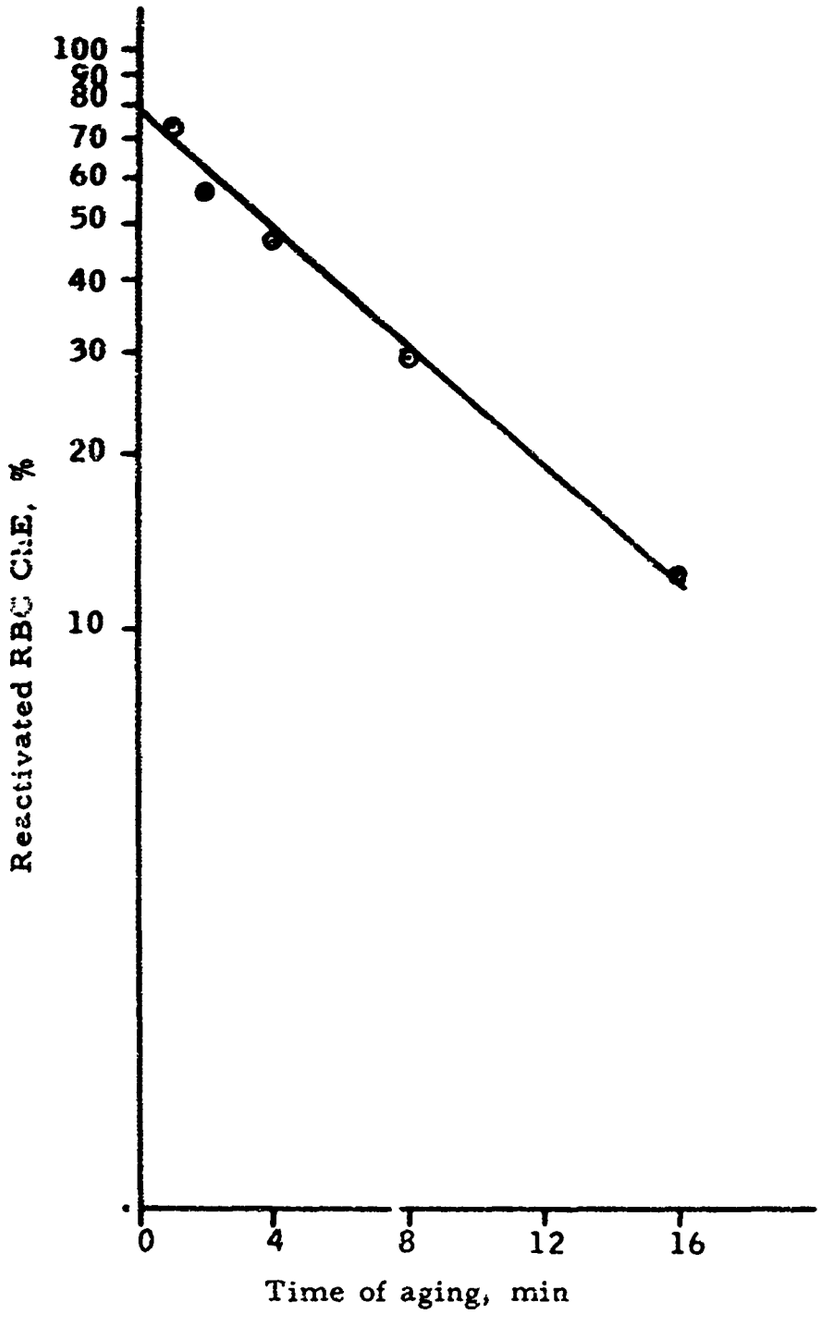


Figure 1. Percentage of Reactivated Dog RBC-ChE Activity as a Function of Time of Aging After Inhibition With Soman in Vitro

The logarithms of the percentage of RBC-ChE activity reactivated by 2-PAMCl, plotted as a function of the time between the injection of soman and sampling into the 2-PAMCl solution, followed first-order kinetics. The mean half times for aging (p = 0.95) and the corresponding rate constants are given in table I.

C. Effect of Varying Concentrations of 2-PAMCl on Dog RBC-ChE Activity Undergoing Aging After Inhibition With Soman in Vitro.

Heparinized dog blood was inhibited with soman at 0°C in borate-buffered 0.9% NaCl at pH 8.8. After centrifugation, the RBC were warmed to 37°C, and aliquots were transferred into the standard 2-PAMCl solution or into phosphate-buffered 0.9% NaCl alone, as described previously. These aliquots were used as controls. Rapid aging was initiated by lowering the pH to 7.35. Then, 2-ml portions of the mixture of RBC and buffer were transferred into the 2-PAMCl solution at 1, 2, and 3 min after the pH adjustment.

At 3.5 min, sufficient concentrated 2-PAMCl solution at pH 7.35 was added to the cell preparation undergoing aging to yield the final concentrations of oxime shown in figure 2. Then, 4-ml portions of the resulting mixture of 2-PAMCl and RBC in phosphate-saline were transferred at various time intervals (up to 1 hr) into an equal volume of 2×10^{-1} M 2-PAMCl to measure the extent of aging of the RBC ChE at the time of transfer. Samples of heparinized dog blood similarly treated but not inhibited by soman were run concurrently. All experimental samples were incubated in the 2-PAMCl solution for 1 hr at 25°C, followed by the washing and centrifugation procedures outlined previously. RBC-ChE activity was measured as described in section III, A.

Representative results, shown in figure 2, indicate that aging of the RBC-ChE activity inhibited by soman was stopped within a few minutes following the addition of 10×10^{-3} M and 5×10^{-3} M concentrations of 2-PAMCl with little further aging. A concentration of 1.25×10^{-3} M also markedly diminished the rate of aging, although the amount of ChE activity remained unaged (that is, reactivatible by the standard 2-PAMCl solution) was less than half that obtained with 10^{-2} M 2-PAMCl. Lower concentrations of 2-PAMCl had little or no effect on the rate of aging or on the reactivation of inhibited, but unaged, enzyme activity (figure 2).

D. ChE Activity of RBC and of Diaphragm and Brain Homogenates of Dogs Given Soman and Treated With 2-PAMCl in Vivo.

The procedures for the preparation of the experimental animals and blood sampling were the same as those in section III, B. Control blood samples were put into standard 2-PAMCl solution or the corresponding phos-

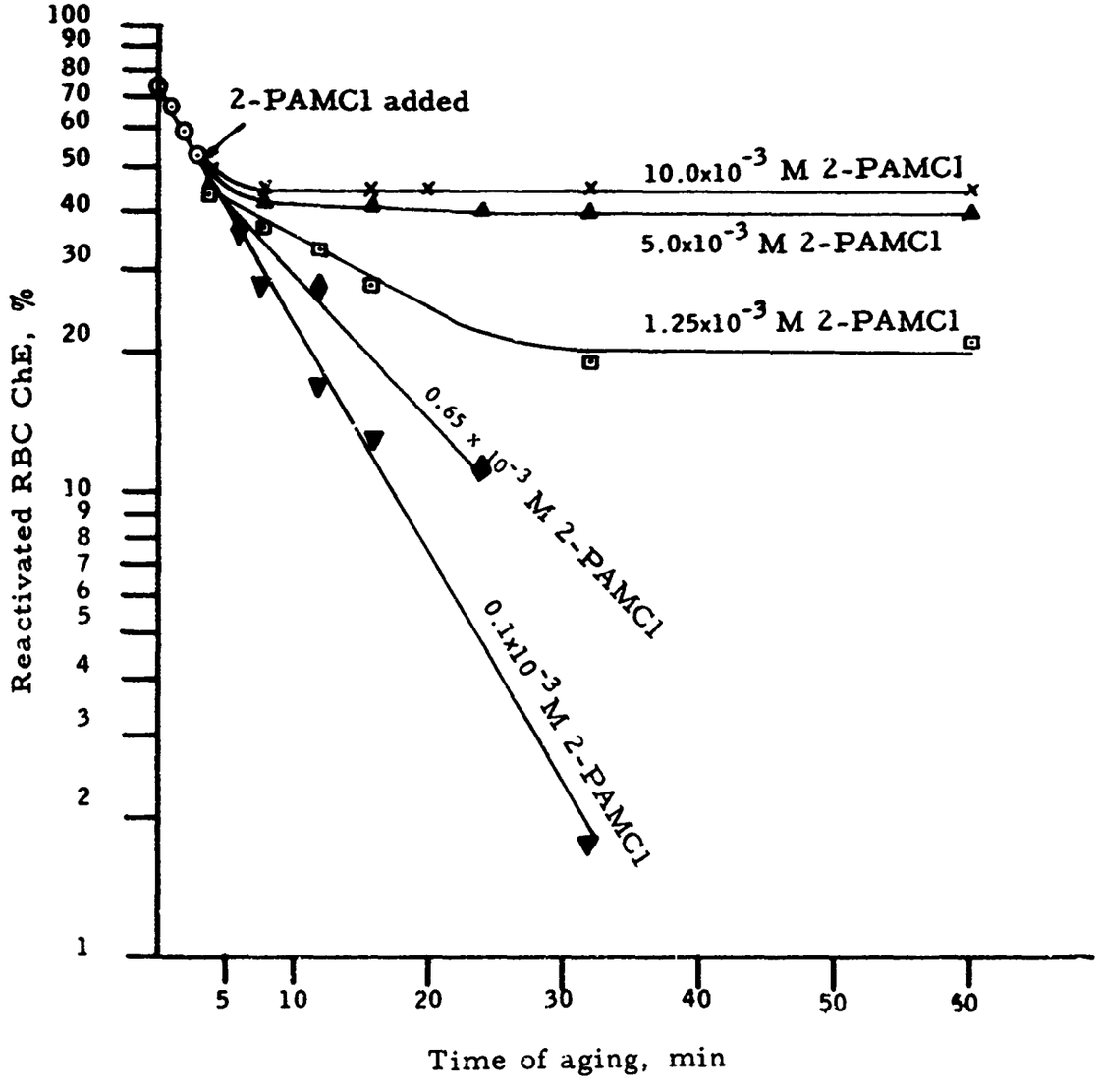


Figure 2. Effect of Varying Concentrations of 2-PAMCl on the Rate of Aging of Dog RBC-ChE Activity After Inhibition With Soman in Vitro

phate-buffered saline; 30 μg/kg of soman was injected, and blood samples were taken at 1, 2, and 3 min to establish the rate of aging. At 3.5 min, 2-PAMCl was injected intravenously via the jugular vein at the following doses (gm/kg): 1 (two dogs), 0.5 (two dogs', 0.25 (two dogs), 0.15 (two dogs), 0.104 (five dogs), and 0.086 (two dogs). Blood samples were taken at various time intervals up to 2 hr after the injection of 2-PAMCl. The administration of 0.104 gm/kg of 2-PAMCl or more resulted in a marked decrease in the rate of aging of RBC-ChE activity in those samples put into the 10^{-1} M 2-PAMCl solution used for measuring changes in reactivatibility (figure 3, curve marked with circles). The RBC-ChE activity remaining in vivo after the dog was poisoned with soman and injected with 2-PAMCl was estimated from the blood samples put into heparinized phosphate-saline (figure 3, curve marked with triangles). The dogs given 1 and 0.5 gm/kg of 2-PAMCl died, either during artificial respiration or upon its termination, as a result of the lethal effects of 2-PAMCl.[10] With doses of 0.25 to 0.086 gm/kg, artificial respiration could be stopped after injection of 2-PAMCl.

The injection of 0.104 gm/kg of 2-PAMCl following poisoning with soman yielded a mean value of 24.0% for the RBC-ChE activity at the end of 2 hr for the five dogs given this dose (table II). Terminal samples of brain and diaphragm were also taken after 2 hr from these dogs. The tissues were homogenized in 0.3 M KCl, and the ChE activity was determined as previously reported.[11]

The results given in table II and those obtained from control animals given a comparable dose of soman but not treated with 2-PAMCl show that significant reactivation is produced in diaphragm muscle as well as in RBC ChE by 2-PAMCl administration under these conditions. The depressed ChE activity found in brain homogenates from dogs poisoned with soman but not treated with 2-PAMCl was not elevated by 2-PAMCl treatment.

IV. DISCUSSION.

Aging of AChE phosphonylated by soman in vitro is minimized by high concentrations of monoisonitrosoacetone (MINA).[7] The effects of varying concentrations of MINA or PAM applied to isolated organs poisoned with soman led Van der Meer and Wolthuis[12] to a similar conclusion independently. However, they stated that "it seems unlikely that the aging rate of a given compound can be substantially affected in the body." Our data permit a different conclusion because a marked diminution in the rate of aging of ChE phosphonylated by soman in vitro (figure 2) and in vivo (figure 3) occurred following 2-PAMCl administration. Such a decrease in the rate of aging following poisoning by an organophosphorus anti-ChE and treatment with 2-PAMCl would contribute to effective therapy because the main effect of oximes, the reactivation

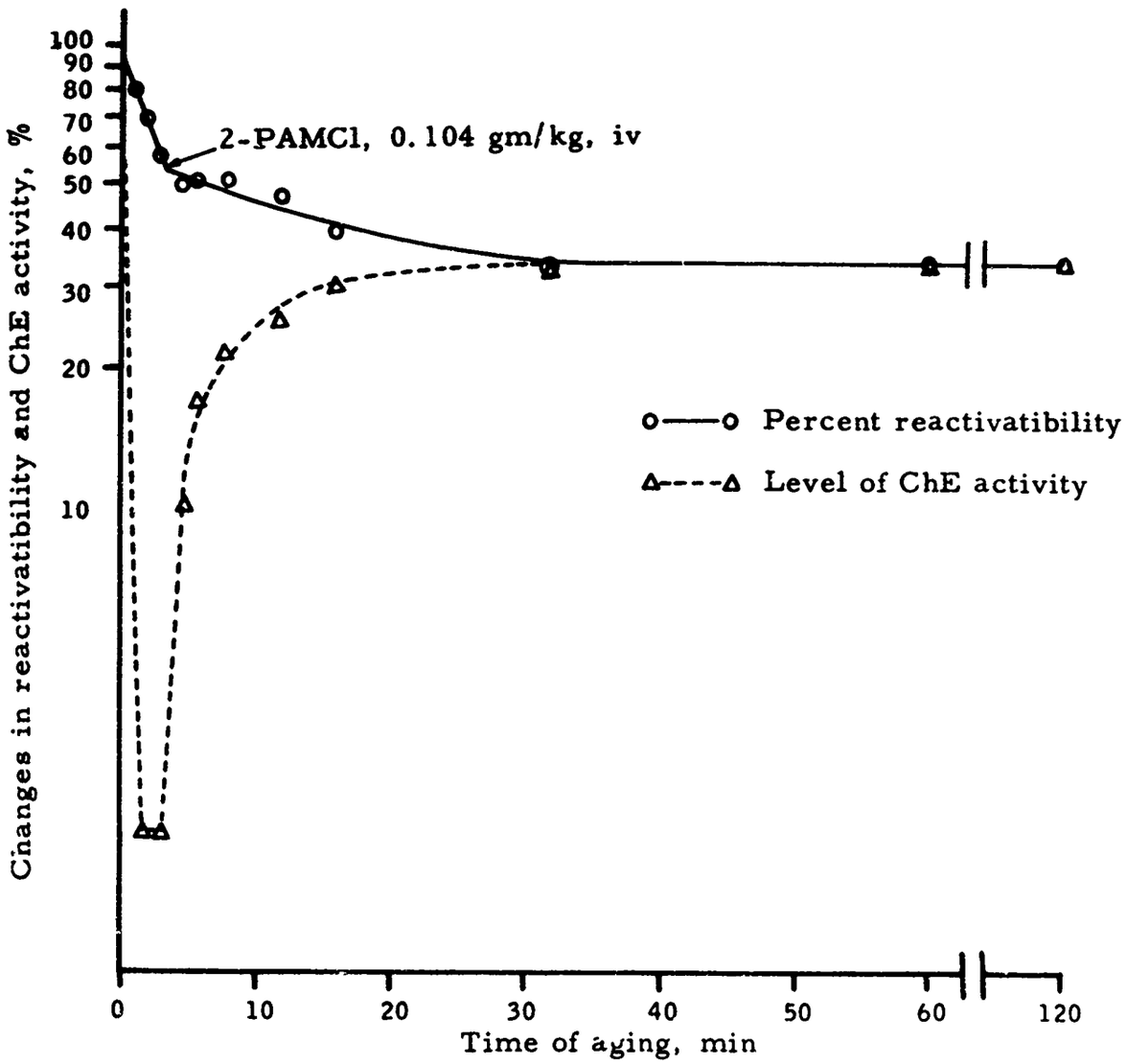


Figure 3. Changes in Reactivatibility and RBC-ChE Activity of Dogs Injected With Soman and Treated With 2-PAMCl in Vivo

of phosphorylated ChE, is limited by the transformation of the enzyme to a nonreactivatible form. In this connection, 2-PAMCl was found to be considerably more potent than MINA in decreasing the rate of aging.*

Table II. ChE Activity a/ of RBC and of Diaphragm and Brain Homogenates of Dogs Poisoned With Soman

Tissue	Amount of acetylcholine hydrolyzed			ChE reactivated
	Control dogs	Dogs poisoned with soman b/	Dogs poisoned with soman b/ and treated with 2-PAMCl	
	μmoles			%
RBC	2.62 ±0.17 c/	0.04 ±0.04	0.63 ±0.18	24.0
Diaphragm	0.90 ±0.10	0.04 ±0.02	0.32 ±0.14	35.6
Brain	1.85 ±0.17	0.10 ±0.07	0.02 ±0.02	0

a/ The values are based upon five control dogs, four dogs poisoned with soman, and five dogs poisoned with soman and treated with 0.104 gm/kg of 2-PAMCl.

b/ Soman, 30 μg/kg, was injected intravenously.

c/ Standard deviation.

The ChE activity of dog RBC inhibited by soman in vivo undergoes rapid aging at a rate not significantly different from that in vitro (table I). The similarity of in vitro and in vivo rates of aging was previously reported for chicken-brain ChE inhibited with either 2,2-dichlorovinyl dimethyl phosphate (DDVP) or S-(1,2-dicarbethoxyethyl)-O, O-dimethyldithiophosphate (malathion)[13] and for sheep RBC ChE and rat-brain ChE inhibited with isopropyl methylphosphonofluoridate (sarin), tetraethyl pyrophosphate (TEPP), DFP, and dimethoxy-p-nitrophenoxy phosphine oxide (DMNP) by Blaber and Creasy.[14]

Furthermore, the depressed ChE activities of the RBC and diaphragm homogenates of dogs poisoned with a single injection of soman were restored to roughly comparable levels by treatment with 2-PAMCl in vivo (table II). This finding suggests that the aging of phosphonylated RBC ChE and diaphragm ChE was approximately equal in rate and in susceptibility to antago-

* Harris, L. W., and Fleisher, J. H. Unpublished observation.

nism by 2-PAMCl. These results and the close correlation between the in vivo and in vitro rates of aging cited above suggest that, in a given species, estimation of the rate of aging of RBC-ChE activity in vitro following inhibition with an organophosphate could determine the time during which oxime therapy would be effective in vivo against intoxication by the same anti-ChE.

Assuming that our results obtained in vitro and in vivo in dogs (tables I and II) are applicable to man,* and when the normal values for RBC-ChE activity are available, the reactivatibility of the ChE in peripheral tissues following a single exposure to an organophosphorus anti-ChE may be roughly approximated from the effect of 2-PAMCl on the inhibited RBC-ChE activity. To obtain this information, a sample of RBC from the exposed individual can be incubated with 2-PAMCl under the conditions described in this report.

When acute exposure to an anti-ChE that yields a rapidly aging phosphorylated ChE is not followed by prompt therapy with 2-PAMCl (in addition to atropine and other appropriate measures[15]), subsequent treatment with 2-PAMCl would probably be of little help. Intravenous injections of high doses of 2-PAMCl, in an effort to restore ChE activity when aging of the inhibited enzyme has gone almost to completion, could be disadvantageous to the poisoned individual because of the detrimental side effects of overdosage.[10,16,17]

No effect on the inhibited brain-ChE activity of dogs poisoned with soman was demonstrable after 2-PAMCl treatment in vivo despite the reactivation by this oxime of RBC-ChE and diaphragm-muscle-ChE activity (table II). This finding is in agreement with earlier observations of the failure of 2-PAMCl to restore the ChE activity of the brain after it was poisoned with other organophosphates[18-20] and is consistent with the inability of 2-PAMCl administration to reverse the central neural effects of organophosphorus anti-ChE poisoning.[21,22]

V. CONCLUSIONS.

The rate of aging of dog RBC ChE inhibited by soman in vitro and in vivo can be markedly diminished by 2-PAMCl, which reactivates the unaged portion of the inhibited ChE enzyme. Measurements of brain- and diaphragm-ChE activity in dogs poisoned with soman and treated with 0.104 gm/kg of

* Because the LD50 of 2-PAMCl in man is unknown, it should be cautioned that the dose of 0.104 gm/kg, which is in excess of 50% of the LD50 in unanesthetized dogs (Crook, J. W., and Cresthull, P. CWLR 2284. The Intravenous Toxicity of Five Oximes in Dogs. 1959. UNCLASSIFIED Report.), is in no way recommended in cases of human poisoning by anti-ChE's.

2-PAMCl showed that reactivation occurred only in the latter tissue. The close correlation found between in vitro and in vivo rates of aging suggests that, in a given species, estimation of the rate of aging of RBC ChE in vitro following inhibition with an organophosphate could determine the time during which oxime therapy would be effective in vivo against intoxication by the same anti-ChE.

LITERATURE CITED

1. Jandorf, B. J., Michel, H. O., Schaffer, N. K., Egan, R., and Summerson, W. H. The Mechanism of Reaction Between Esterases and Phosphorus-Containing Anti-esterases. Discussions Faraday Soc. 20, 134-142 (1955).

2. Hobbiger, F. Effect of Nicotinhydroxamic Acid Methiodide on Human Plasma Cholinesterase Inhibited by Organophosphates Containing a Dialkylphosphoro Group. Brit. J. Pharmacol. 10, 356-362 (1955).

3. Wilson, I. B. Promotion of Acetylcholinesterase Activity by the Anionic Site. Discussions Faraday Soc. 20, 119-125 (1955).

4. Berends, F., Posthumus, C. H., van der Sluys, I., and Deierkauf, F. A. The Chemical Basis of the "Aging Process" of DFP-Inhibited Pseudocholinesterase. Biochem. Biophys. Acta 34, 576-578 (1959).

5. Green, A. L., and Saville, B. The Reaction of Oximes With Isopropyl Methylphosphonofluoridate (Sarin). J. Chem. Soc., 3887-3892 (1956).

6. Loomis, T. A., and Salafsky, B. Antidotal Action of Pyridinium Oximes in Anticholinesterase Poisoning; Comparative Effects of Soman, Sarin, and Neostigmine on Neuromuscular Function. Toxicol. Appl. Pharmacol. 5, 685-701 (1963).

7. Fleisher, J. H., and Harris, L. W. Dealkylation as a Mechanism for Aging of Cholinesterase After Poisoning With Pinacolyl Methylphosphonofluoridate. Biochem. Pharmacol. 14, 641-650 (1965).

8. Fleisher, J. H., Pope, E. J., and Spear, S. F. Determination of Red Blood Cell Cholinesterase Activity in Whole Blood. An Application of the Colorimetric Method to the Blood of the Rabbit, Rat, Pig, Dog, Goat, and Monkey. A. M. A. Arch. Ind. Health 11, 332-337 (1955).

9. Hobbiger, F. Chemical Reactivation of Phosphorylated Human and Bovine True Cholinesterases. Brit. J. Pharmacol. 11, 295-303 (1956).

10. Davies, D. R., and Willey, G. L. The Toxicity of 2-Hydroxyiminomethyl-N-methylpyridinium Methanesulphonate (P2S). Ibid. 13, 202-206 (1958).

11. Fleisher, J. H., Hansa, J., Killos, P. J., and Harrison, C. S. Effects of 1,1-Trimethylene Bis(4-formylpyridinium Bromide) Dioxime (TMB-4) on Cholinesterase Activity and Neuromuscular Block Following Poisoning With Sarin and DFP. J. Pharmacol. Exptl. Therap. 130, 461-468 (1960).

12. Van der Meer, C., and Wolthuis, O. L. The Effect of Oximes on Isolated Organs Intoxicated With Organo-phosphorus Anticholinesterases. Biochem. Pharmacol. 14, 1299-1312 (1965).

13. Witter, R. F., and Gaines, T. B. Rate of Formation in Vivo of the Unreactivatable Form of Brain Cholinesterase in Chickens Given DDVP or Malathion. Ibid. 12, 1421-1427 (1963).

14. Blaber, L. C., and Creasy, N. H. The Mode of Recovery of Cholinesterase Activity in Vivo After Organophosphorous Poisoning. Biochem. J. 77, 591-596 (1960).

15. Grob, D. Anticholinesterase Intoxication in Man and Its Treatment. In: Cholinesterases and Anticholinesterases Agents. Koelle, G. B., ed. pp 990-1027. Springer-Verlag, Berlin, Germany. 1963.

16. Jager, B. V., and Stagg, G. N. Toxicity of Diacetyl Monoxime and of Pyridine-2-aldoxime Methiodide in Man. Johns Hopkins Hosp. Bull. 102, 203-211 (1958).

17. Fleisher, J. H., Moen, T. H., and Ellingson, N. R. Effects of 2-PAM and TMB-4 on Neuromuscular Transmission. J. Pharmacol. Exptl. Therap. 149, 311-319 (1965).

18. Hobbiger, F. Protection Against the Lethal Effects of Organophosphates by Pyridine-2-aldoxime Methiodide. Brit. J. Pharmacol. 12, 438-446 (1957).

19. Kewitz H., and Nachmansohn, D. A Specific Antidote Against Lethal Alkylphosphate Intoxication. IV. Effects in Brain. Arch. Biochem. 66, 271-283 (1957).

20. Rutland, J. P. The Effect of Some Oximes in Sarin Poisoning. Brit. J. Pharmacol. 13, 399-403 (1958).

21. Grob, D., and Johns, R. J. Use of Oximes in the Treatment of Intoxication by Anticholinesterase Compounds in Normal Subjects. Am. J. Med. 24, 497-511 (1958).

22. Wills, J. H., and Borison, H. L. Modification by Sarin and Antagonists of Medullary Respiratory Activities. Federation Proc. 18, 459 (1959).

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) US Army Edgewood Arsenal ATTN: SMUEA-RMP Edgewood Arsenal, Maryland 21010	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP N/A

3. REPORT TITLE
REACTIVATION BY 2-PAMCl OF INHIBITED ChE ACTIVITY IN DOGS POISONED WITH PINACOLYL METHYLPHOSPHONOFLUORIDATE (SOMAN, GD)

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
This work was started in April 1964 and completed in May 1965.

5. AUTHOR(S) (Last name, first name, initial)
Fleisher, Joseph H., Harris, Larrel W., and Murtha, Edmund F.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
January 1967	35	22

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	EATR 4053
c. Task No. 1C622401A09709	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) N/A
d.	

10. AVAILABILITY/LIMITATION NOTICES
Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
Prophylaxis and therapy of agent GD	N/A

13. ABSTRACT
(U) In this work, the effect of varying concentrations and dosages of 2-pyridine aldoxime methochloride (2-PAMCl) on the aging of cholinesterase (ChE) phosphonylated by soman in vitro and in vivo in dogs was studied. The rate of aging of dog red-blood-cell (RBC) ChE inhibited by soman in vitro and in vivo can be markedly diminished by 2-PAMCl, which reactivates the unaged portion of the inhibited ChE enzyme. Measurements of brain- and diaphragm-ChE activity in dogs poisoned with soman and treated with 0.104 gm/kg of 2-PAMCl showed that reactivation occurred only in the latter tissue. The close correlation found between in vitro and in vivo rates of aging suggests that, in a given species, estimation of the rate of aging of RBC ChE in vitro following inhibition with an organophosphate could determine the time during which oxime therapy would be effective in vivo against intoxication by the same anti-ChE.

14. KEYWORDS

Cholinesterase	Organophosphates	In vivo
Pinacolyl methylphosphonofluoridate	GD	Treatment
Soman	Erythrocyte ChE	Therapy
2-Pyridine	Brain ChE	2-PAMCl
Aging	Diaphragm ChE	
Reactivation	In vitro	

DD FORM 1 JAN 64 1473

UNCLASSIFIED
Security Classification